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## Original Research Article

## Anti-hyperlipidemic effects of *Caralluma edulis* (Asclepiadaceae) and *Verbena officinalis* (Verbenaceae) whole plants against high-fat diet-induced hyperlipidemia in mice

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### Abstract

**Purpose:** To investigate the anti-hyperlipidemic effect of *Caralluma edulis* and *Verbena officinalis*.

**Methods:** Phytochemical analysis of crude extracts of *Caralluma edulis* (Ce.Cr) and *Verbena officinalis* (Vo.Cr) were carried out. Hyperlipidemia was induced in mice with high-fat diet (HFD, 1.25 % w/w cholesterol, 0.5 % w/w cholic acid and 10 % v/w coconut oil). All the groups, except the saline-treated group, were fed on HFD for 4 weeks (lead-in period) to induce hyperlipidemia. Thereafter, the groups were treated with varying doses of the plant extract for 2 weeks (treatment period) as well as atorvastatin (10 mg/kg) reference standard. Body weight was measured fortnightly for all groups. Total cholesterol (TC), triglyceride (TGs) and low density lipoprotein (LDL) were assayed using Merck diagnostic kits. For histopathological analysis, liver slices were fixed in 10 % formalin and embedded in paraffin wax and was examined with the aid of hematoxylin and eosin staining (H & E).

**Results:** *Caralluma edulis* (Ce.Cr) contains saponins, alkaloids, tannins, phenol, glycosides, terpenoids and flavonoids while *Verbena officinalis* (Vo.Cr) tested positive for the presence of alkaloids, carbohydrates, flavonoids, saponins and tannins. HFD increased total cholesterol (TC), triglyceride (TGs), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) compared to regulator diet ( $p < 0.001$ ). Treatment of the animals with Ce.Cr and Vo.Cr dose-dependently (500 - 1000 mg/kg) reduced serum TC, TGs, LDL and VLDL ( $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$ , vs. HFD group) and raised high density lipoprotein (HDL) ( $p < 0.01$ , vs. HFD group), similar to that observed with atorvastatin (10 mg/kg). The anti-hyperlipidemic effects of Ce.Cr and Vo.Cr were also confirmed via liver histopathology results, showing improved structure with no hepatocellular necrosis and fat accumulation.

**Conclusion:** These results indicate that *Caralluma edulis* and *Verbena officinalis* exhibit anti-hyperlipidemic effect; thus, the plants have therapeutic potentials for the management of lipid disorders.

**Keywords:** *Caralluma edulis*, *Verbena officinalis*, Anti-hyperlipidemia, Hepatocellular necrosis

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### INTRODUCTION

Hyperlipidemia leads to the progression of various cardiovascular complications, such as

atherosclerosis, myocardial infarction and hypertension [1]. Various types of lipids such as total cholesterol (TC), triglyceride (TGs), high density lipoprotein (HDL), and low density

lipoprotein (LDL), very low density lipoproteins (VLDL) cause various health problems such as pancreatitis and cardiovascular disorders [2]. It has been reported that about one fourth of the people suffering from myocardial infarction had hyperlipidemia [3]. Effective management of hyperlipidemia reduces the risk of cardiovascular events. Current therapy for hyperlipidemia management includes statins, fibrates, and bile acid sequestrates but the use of these drugs has been associated with many side effects. Medicinal plants enriched in phytochemicals such as tannins, saponins, flavonoids, essential oils and alkaloids have curative properties and are used in the traditional system of medicine for the management of various ailments [4]. Oxidative stress plays an important role in the onset of hyperlipidemia due to free radical generation, which leads to the further progression of cardiovascular diseases [5]. Medicinal plants being rich in antioxidant constituents possess profound antioxidant properties.

*Caralluma edulis* commonly known as "Choung" belongs to family Asclepiadaceae. Genus *Caralluma* enriched in pregnane glycosides, megastigmane glycosides, flavone and esters [6]. *Caralluma edulis* is known to possess antidiabetic [7] and antioxidant [8] properties.

*Verbena officinalis* commonly known as "Vervian", "Herb of grace" belongs to family Verbenaceae [9]. *Verbena officinalis* contain various irridoids such as verbenalin and hastatoside which possess antioxidant properties [10]. In the current investigation we evaluated anti-hyperlipidemic activity of *Caralluma edulis* and *Verbena officinalis* using high fat diet (HFD) animal model to explore their use in the treatment of hyperlipidemia.

## EXPERIMENTAL

### Plant material and extraction

The whole plant *Caralluma edulis* was purchased from a local market in May and the whole plant of *Verbena officinalis* was collected from Rawat, Islamabad, Pakistan. The plant samples were identified from Dr. Mushtaq Ahmad at Plant Sciences Department, Quaid-i-Azam University, Islamabad. The sample specimen bearing voucher number ISB-422 and ISB-270 were deposited to the herbarium of same department. The plants were thoroughly washed, shade dried at room temperature and coarsely ground. The *Caralluma edulis* powdered material (3 kg) was treated with 80 % aqueous-ethanol for seven days and *Verbena officinalis* grinded powder (2.7

kg) was soaked in 70 % methanol for 14 days, with occasional shaking and mixing. Both the plant materials were filtered through grade 1 Whatmann filter paper [11]. The filtered materials were concentrated on rotary evaporator to obtain a thick semi-solid paste i.e. *Caralluma edulis* crude extract (Ce.Cr) and *Verbena officinalis* crude extract (Vo.Cr), yielding approximately 4.67 % (w/w) and 18.66 % (w/w) respectively. The crude extracts were solubilized in normal saline.

### Chemicals

Cholic acid and cholesterol were purchased from Sigma Chemicals (Germany), atorvastatin from Getz Pharmaceuticals, Karachi Pakistan and crude coconut oil procured from Marhaba lab, Pvt Ltd. Lahore. Analytical grade chemicals were used. TC, TGs and HDL diagnostic kits were purchased from Merck (Japan).

### Animals

Balb-C mice (25 - 35 g) were used in the study and placed at Animal House of the Riphah Institute of Pharmaceutical Sciences, where controlled environmental conditions were maintained at  $25 \pm 2^{\circ}\text{C}$  temperature, 12 h light and dark cycle with free access to standard diet and tap water *ad libitum*. Animal experiments were performed in accordance with "Principles of Laboratory Animal care" (NIH publication 85-23, revised 1985) [12] approved by Ethics Committee of Riphah Institute of Pharmaceutical Sciences (ref. no. REC/RIPS/2016/001).

### Phytochemical characterization

The phytochemical analysis was performed according to the standard protocols [13] with slight modifications.

### Induction of hyperlipidemia

Hyperlipidemia was induced by feeding on high fat diet (1.25 % w/w cholesterol, 0.5 % w/w cholic acid and 10 % v/w coconut oil with slight modifications [14]. The period of 4 week was considered as the lead-in period to induce hyperlipidemia in mice while in the next two week period treatment was done with different doses of plant. Body weights were measured after every two week for all groups.

### Anti-hyperlipidemic activity

Balb-C mice were divided into seven groups each consisting of six animals. Group 1 served as regular diet control treated orally with saline

(10 mL/kg). Group 2 received only HFD. Group 3 and 4 received Ce.Cr at the dose of 500 and 1000 mg/kg respectively along with HFD. Group 5 and 6 received Vo.Cr at the dose 500 and 1000 mg/kg respectively along with HFD. Group 7 received atorvastatin dose 10 mg/kg with HFD. All the doses were given through oral route (p.o).

### Determination of hyperlipidemia

The blood sample (1 mL) was collected using disposable syringe via cardiac puncture and allowed to clot at room temperature in sterile gel tubes for 45 min. The plasma was separated through centrifugation at 3000 rpm for 10 min and the samples were analyzed spectrophotometrically. TC, TGs and HDL were assayed using Merck diagnostic kits. LDL and VLDL concentrations were measured using Friedewald's equations as in Eqs 1 and 2, respectively [15].

$$\text{VLDL} = (\text{TGs})/5 \dots\dots\dots (1)$$

$$\text{LDL} = \text{TC} - (\text{HDL}) - (\text{VLDL}) \dots\dots\dots (2)$$

### Body weight measurement

Body weight was measured initially, then after every two weeks for all the groups.

### Histopathological analysis

Liver slices were fixed in 10 % formalin and embedded in paraffin wax and histopathological analysis of liver samples studied through Hematoxylin and Eosin staining (H & E).

### Statistical analysis

Data expressed as Mean  $\pm$  SEM (standard error of mean). Results were analyzed using one-way analysis of variance (ANOVA) followed by post-hoc Tukey test.  $P < 0.05$  was considered significant.

## RESULTS

### Phytochemical profile

The phytochemical analysis revealed that Ce.Cr contains saponins, alkaloids, tannin, phenol, glycosides, terpenoids and flavonoids. While Vo.Cr indicates the presence of for alkaloids, carbohydrates, flavonoids, saponins and tannins.

### Effect of Ce.Cr and Vo.Cr on TC

Ce.Cr and Vo.Cr dose-dependently (500 - 1000 mg/kg) reduce the HFD-induced raise in level of TC (Table 1). In saline treated group, TC value was  $92.8 \pm 8.66$  mg/dL. HFD treatment raised level of TC to  $386.2 \pm 21.82$  mg/dL ( $p < 0.001$  vs. saline group). Treatment of groups with Ce.Cr at the dose of 500 and 1000 mg/kg for two weeks reduced TC level to  $305.4 \pm 6.72$  and  $287.8 \pm 16.85$  mg/dL ( $p < 0.01$ ;  $p < 0.001$  vs. HFD group) respectively. Similarly Vo.Cr at 500 and 1000 mg/kg reduced TC level from  $386.2 \pm 21.82$  mg/dL to  $165 \pm 14.47$  and  $116.8 \pm 9.82$  mg/dL ( $p < 0.001$ ;  $p < 0.001$  vs. HFD group) respectively. Atorvastatin (10 mg/kg) reduces TC level to  $161.4 \pm 9.47$  mg/dL ( $p < 0.001$  vs. HFD group).

### Effect of Ce.Cr and Vo.Cr on TGs

Ce.Cr and Vo.Cr dose-dependently (500 - 1000 mg/kg) reduced the HFD induced increased level of TGs (Table 1). TGs value of saline treated group was  $86.6 \pm 6.85$  mg/dL. Treatment of mice with HFD raised level of TGs to  $162 \pm 14.21$  mg/dL ( $p < 0.001$  vs. saline group). Ce.Cr at doses of 500 and 1000 mg/kg reduced TGs level to  $139.2 \pm 11.35$  mg/dL and  $125.4 \pm 6.72$  mg/dL respectively ( $p < 0.05$  vs. HFD group) as shown in Table 1, Vo.Cr (500 and 1000 mg/kg) caused significant reduction in the HFD-induced increased level of TGs in rats plasma. In atorvastatin (10 mg/kg) treated group, TGs level value was  $114.4 \pm 8.08$  mg/dL ( $p < 0.05$  vs. HFD group).

**Table 1:** Inhibitory effect of *Caralluma edulis* crude extract (Ce.Cr) and *Verbena officinalis* crude extract (Vo.Cr) against high fat diet (HFD)-induced increase in total cholesterol (TC), triglycerides (TGs), high density lipoprotein (HDL) and low density lipoprotein (LDL) in mice

Treatment	TC (mg/dL)	TGs (mg/dL)	HDL(mg/dL)	LDL (mg/dL)
Saline (10 mL/kg)	$92.8 \pm 8.66$	$86.6 \pm 6.85$	$33.4 \pm 2.16$	$41.6 \pm 7.09$
HFD	$386.2 \pm 21.82^{\#}$	$162 \pm 14.21^{\#}$	$32 \pm 2.608$	$317.4 \pm 23.37^{\#}$
HFD + Ce.Cr (500 mg/kg)	$305.4 \pm 6.72^{**}$	$139.2 \pm 11.35^{**}$	$51.2 \pm 2.26^{**}$	$229.6 \pm 6.34^{**}$
HFD + Ce.Cr (1000 mg/kg)	$287.8 \pm 16.85^{***}$	$125.4 \pm 6.72^{**}$	$51.8 \pm 3.08^{**}$	$210.6 \pm 18.53^{***}$
HFD + Vo.Cr (500 mg/kg)	$165 \pm 14.47^{***}$	$148 \pm 10.83^{**}$	$49 \pm 0.83^{**}$	$86 \pm 15.64^{***}$
HFD + Vo.Cr (1000 mg/kg)	$116.8 \pm 9.82^{***}$	$82.8 \pm 6.39^{***}$	$50.8 \pm 0.66^{**}$	$49 \pm 9.36^{***}$
HFD + Atorvastatin (10 mg/kg)	$161.4 \pm 9.47^{***}$	$114.4 \pm 8.08^{**}$	$49 \pm 1.51^{**}$	$89 \pm 9.70^{***}$

Values are expressed as Mean  $\pm$  SEM (n = 5);  $^{\#}p < 0.001$  vs. saline group,  $^{**}p < 0.01$ ,  $^{***}p < 0.001$  vs. HFD treated group; one-way ANOVA with Tukey post-hoc test

### Effect of Ce.Cr and Vo.Cr on HDL

Ce.Cr and Vo.Cr increased the level of HDL in dose-dependent (500 - 1000 mg/kg) manner (Table 1). The HDL level of saline treated group was  $33.4 \pm 2.16$  mg/dL. In HFD treatment group, the level of HDL was  $32 \pm 2.608$  mg/dL ( $p > 0.05$  vs. saline group). Ce.Cr at dose of 500 and 1000 mg/kg caused significant ( $p < 0.01$  vs. HFD group) increase in the HDL level to  $51.2 \pm 2.26$  mg/dL and  $51.8 \pm 3.08$  mg/dL respectively ( $p < 0.01$  vs. HFD group). Similar results were obtained in groups treated with Vo.Cr (500 and 1000 mg/kg). In atorvastatin (10 mg/kg) treated group, HDL level value was  $49 \pm 1.51$  mg/dL ( $p < 0.01$  vs. HFD group).

### Effect of Ce.Cr and Vo.Cr on LDL

Ce.Cr and Vo.Cr dose-dependently (500 - 1000 mg/kg) reduced the HFD-induced raise in level of LDL (Table 1). In saline treated group, LDL value was  $41.6 \pm 7.09$  mg/dL. HFD treatment raised level of LDL to  $317.4 \pm 23.37$  mg/dL ( $p < 0.001$  vs. saline group). Ce.Cr (500 and 1000 mg/kg) reduced LDL level to  $229.6 \pm 6.34$  mg/dL ( $p < 0.01$  vs. HFD group) and  $210.6 \pm 18.53$  mg/dL ( $p < 0.001$  vs. HFD group) respectively. Vo.Cr (500 and 1000 mg/kg) produced marked reduction in the LDL level to  $86 \pm 15.64$  mg/dL ( $p < 0.001$  vs. HFD group) and  $49 \pm 9.36$  mg/dL ( $p < 0.001$  vs. HFD group) respectively. Similarly atorvastatin (10 mg/kg) produced significant reduction in the LDL level up to  $89 \pm 9.70$  mg/dL ( $p < 0.001$  vs. HFD group).

### Effect of Ce.Cr and Vo.Cr on VLDL

Ce.Cr and Vo.Cr dose-dependently (500 - 1000 mg/kg) reduced the HFD induced raised in level of VLDL (Table 2). In saline treated group, VLDL value was  $17 \pm 1.44$  mg/dL. HFD treatment raised level of VLDL to  $32 \pm 2.90$  mg/dL ( $p < 0.001$  vs. saline group). In Ce.Cr (500 and 1000 mg/kg) treated groups, VLDL level reduced to  $27.4 \pm 2.20$  mg/dL and  $24.6 \pm 1.28$  mg/dL ( $p < 0.05$  vs. HFD group) respectively. Vo.Cr at dose

of 500 and 1000 mg/kg decreased VLDL level to  $29.2 \pm 2.22$  mg/dL ( $p < 0.05$  vs. HFD group) and  $16 \pm 1.30$  mg/dL ( $p < 0.001$  vs. HFD group) respectively. Atorvastatin (10 mg/kg) reduced the VLDL level to  $22.4 \pm 1.63$  mg/dL ( $p < 0.05$  vs. HFD group).

**Table 2:** Inhibitory effect of *Caralluma edulis* crude extract (Ce.Cr) and *Verbena officinalis* crude extract (Vo.Cr) against high fat diet (HFD)-induced increase in very low density lipoprotein (VLDL) in mice

Treatment	VLDL (mg/dL)
Saline (10 mL/kg)	$17.00 \pm 1.44$
HFD	$32.00 \pm 2.90^{\#}$
HFD + Ce.Cr (500 mg/kg)	$27.40 \pm 2.20^*$
HFD + Ce.Cr (1000 mg/kg)	$24.60 \pm 1.28^*$
HFD + Vo.Cr (500 mg/kg)	$29.20 \pm 2.22^*$
HFD + Vo.Cr (1000 mg/kg)	$16.00 \pm 1.30^{***}$
HFD + Atorvastatin (10 mg/kg)	$22.40 \pm 1.63^*$

Values expressed as mean  $\pm$  SEM ( $n = 5$  in each group);  $^{\#}p < 0.001$  vs. saline group,  $^*p < 0.05$ ,  $^{***}p < 0.001$  vs. HFD treated group, one-way ANOVA with Tukey post-hoc test

### Effect of Ce.Cr and Vo. Cr on body weight

Ce.Cr at 500 and 1000 mg/kg reduced the body weight of mice, while Vo.Cr at 500 and 1000 mg/kg doses does not cause any reduction of animal body weight at end of treatment as shown in Table 3.

### Histopathological features

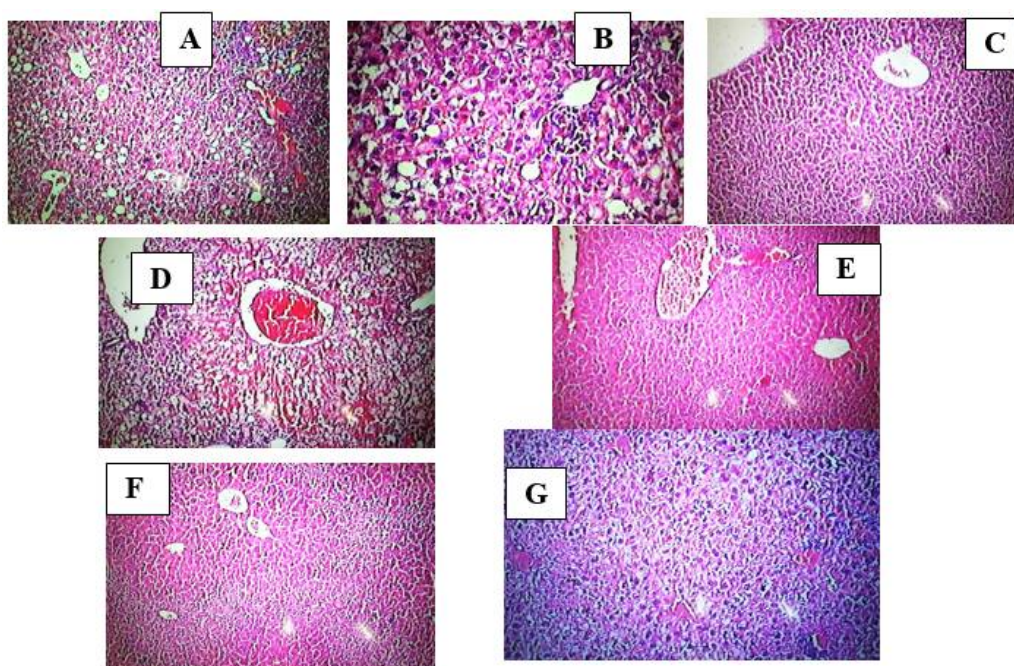
Hematoxylin and eosin staining clearly showed fatty liver of mice fed on HFD compared to the animals fed on regular diet. Deposition of fat in mice fed on HFD was decreased in animals treated with Ce.Cr (500 and 1000 mg/kg). As shown in Figure 1, fat deposition was not seen in animals treated with Vo.Cr (500 and 1000 mg/kg) and atorvastatin (10 mg/kg).

The liver section of mice treated with saline group shows no fatty changes and composed of central vein surrounded by hepatic cords (Figure 1A). In HFD group, the liver showed central vein congestion and marked fatty change (Figure 1B).

**Table 3:** Effect of *Caralluma edulis* crude extract (Ce.Cr) and *Verbena officinalis* crude extract (Vo.Cr) on the body weight of high fat diet (HFD) administered mice

GROUPS	Body weight (g)			
	0 week	2 <sup>nd</sup> week	4 <sup>th</sup> week	6 <sup>th</sup> week
Saline (10 mL/kg)	$29.3 \pm 1.17$	$29.6 \pm 1.15$	$29.9 \pm 1.09$	$30.5 \pm 1.19$
HFD	$31.1 \pm 1.09$	$32.0 \pm 0.84$	$33.6 \pm 0.81$	$35.1 \pm 1.33$
HFD + Ce.Cr (500 mg/kg)	$29.7 \pm 0.55$	$30.8 \pm 0.72$	$31.4 \pm 0.66$	$30.1 \pm 0.86$
HFD + Ce.Cr (1000 mg/kg)	$29.6 \pm 0.69$	$30.3 \pm 0.75$	$30.6 \pm 0.70$	$29.2 \pm 0.40$
HFD + Vo.Cr (500 mg/kg)	$31.4 \pm 0.80$	$31.9 \pm 0.63$	$32.4 \pm 0.71$	$32.8 \pm 0.78$
HFD + Vo.Cr (1000 mg/kg)	$32.6 \pm 0.59$	$33.2 \pm 0.65$	$33.8 \pm 0.70$	$34.5 \pm 0.46$
HFD + Atorvastatin (10mg/kg)	$31.2 \pm 0.88$	$32.4 \pm 0.70$	$33.0 \pm 0.71$	$33.3 \pm 0.72$

Values expressed as mean  $\pm$  SEM ( $n = 5$ )



**Figure 1:** Representative micrographs (40x magnification, hematoxylin and eosin staining), showing effect of *Caralluma edulis* crude extract (Ce.Cr) and *Verbena officinalis* crude extract (Vo.Cr) against HFD induced histopathological changes in mice liver. **A.** Saline (10 mL/kg) treated liver tissue, showing normal central vein surrounded by hepatic cords with no fatty change. **B.** HFD intoxicated liver tissue, showing central vein congestion and marked fatty change. **C.** Ce.Cr (500 mg/kg) treated liver tissue, showing central vein congestion and moderate fatty change. **D.** Ce.Cr (1000 mg/kg) treated liver tissue, showing central vein congestion and mild fatty change. **E.** Vo.Cr (500 mg/kg) treated liver showing portal vein congestion and periportal hepatocytes drop out with no fatty change. **F.** Vo.Cr (1000 mg/kg) treated liver showing periportal hepatocytes drop out with no fatty change. **G.** Atorvastatin (10 mg/kg) treatment showing portal vein congestion and hepatocytes drop out with no fatty change.

In the Ce.Cr (500 mg/kg) treated animals, the liver tissue showed central vein congestion and moderate fatty change (Figure 1C).

Animals treated with a higher dose of Ce.Cr (1000 mg/kg) showed liver tissue with central vein congestion and mild fatty change (Figure 1D). In the Vo.Cr (500 mg/kg) treated animals the liver tissue showed periportal hepatocytes drop out, portal vein congestion with no fatty change (Figure 1E). Similar effects were observed with higher dose of Vo.Cr (1000 mg/kg), Figure 1F. Atorvastatin (10 mg/kg) treated group showed central vein congestion and no fatty change (Figure 1G).

## DISCUSSION

This study demonstrates the anti-hyperlipidemic effect of *Caralluma edulis* and *Verbena officinalis* crude extract. Treatment with Ce.Cr extract and Vo.Cr extract lowered lipid profile and raised the HDL in mice. HFD-induced hyperlipidemia in mice causes increase in plasma TC, TGs, LDL, VLDL and oxidative damage in liver [16]. HFD induced hyperlipidemia decreases  $\beta$ -oxidation and increases the synthesis of cholesterol and oxidative stress due to decreased free radical

scavenger enzymatic gene expression. Plant polyphenols plays a preventive role in cardiovascular diseases including atherosclerosis. Combination of 0.25 - 0.5 % cholic acid with cholesterol increases cholesterol absorption and produces atherosclerosis in normal mice [17]. Cholic acid plays an important role in liver inflammation and lowers the production of bile acid and affects the TGs and HDL levels which further leads to development of atherosclerosis. Different types of fats are used to provide energy (20 – 60 %) derived from either using animal fats (beef tallow or lard) or plant fats oils (coconut or olive oil).

Coconut oil, lard and olive oil cause hepatic steatosis without inflammation [18]. Mice fed with HFD increased the body weight compared to standard diet. HFD induced hepatic steatosis and inflammation begins with presence of leukocytes in the tissue. Hypercholesterolemia causes increase in the production of free radicals and raises levels of lipid peroxides [19]. Myristic acid and lauric acids are present in coconut oil; they increase level of LDL more than HDL. The elevation of the LDL level in animals fed on HFD could be related to changes in hepatic LDL



receptors. It also causes oxidative stress that further leads to increased oxidized LDL levels.

Stress due to oxidation plays a very magnificent role in the development of cardiac complication, neurodegenerative diseases, and cancer and in the aging process. Flavonoids and terpenoids may reduce TC, TGs, LDL and VLDL through the inhibition of pancreatic lipases which are responsible for the conversion of triglyceride into fatty acids and glycerol [20]. Plants containing polyphenols inhibit lipid peroxidation and increases glutathione peroxidase which prevents tissue damage by neutralizing reactive oxygen species [21]. The total phenolic content of *Verbena officinalis* was  $652.50 \pm 2.36$  Gallic Acid Equivalent (GAE g/kg dry mass). The total flavonoids contents were  $188.90 \pm 2.5$  quercetin equivalents (QE g/kg of dry mass).

The phenolic and flavonoid contents of these plants might have contributed to the observed beneficial effects on lipid profile in animals fed on HFD. The antioxidant inhibitory concentration was  $15.76 \pm 0.8$  mg/L compared to vitamin C  $4.4 \pm 0.2$  mg/L respectively. The anti-hyperlipidemic effect exhibited by *Caralluma edulis* may be related to its known antioxidant potential and in *Verbena officinalis* may be due to the presence of phytochemical compounds such as flavonoids, phenolic compounds, terpenoids, phenyl propanoids and iridoids, which were reported to possess radical scavenging activity [22].

## CONCLUSION

The current study reveals that *Caralluma edulis* and *Verbena officinalis* crude extracts possess anti-hyperlipidemic potential against HFD-induced hyperlipidemia. Further advanced studies were needed to identify the active principles accounting for anti-hyperlipidemic effect and to elucidate the possible mechanism of action.

## DECLARATIONS

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### Conflict of Interest

No conflict of interest associated with this work.

## Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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